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TWO-COMPONENT STRUCTURE OF CATALYST FOR NITROGEN FIXATION IN AZOTOBACTER; PHYSIOLOGICAL COURSE OF MOLECULAR NITROGEN FIXATION

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[A Digest]

Experimental data in the author's previous work made it possible to determine that the protoplasm in cells of Azotobacter contains a specific catalyst (ferment) which takes care of the fixation of molecular nitrogen. This catalyst's molecule was found to contain an amino, a carboxyl, and carbonyl group. Only one of these three groups, the carboxyl, takes part directly in the fixation of atmospheric nitrogen. On the basis of this data, the author developed a preliminary scheme explaining the process of fixation in which it was assumed that the molecular nitrogen reacts with the hydrated carbonyl groups. However, investigation of ascorbic acid's influence on fixation of nitrogen by frozen cells of the Azotobacter, showed that two adjacent carbonyl groups participated in the fixation process as follows:

$$\begin{array}{l} R - \underset{\underset{O}{\|}}{C} - \underset{\underset{O}{\|}}{C} - R_1 + N_2 \rightarrow R - \underset{\underset{\underset{N}{|}}{\underset{O}{|}}}{C} = \underset{\underset{\underset{N}{|}}{\underset{O}{|}}}{C} - R_1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad N = N \end{array}$$

Considering the previously determined presence of protein in the catalyst for nitrogen fixation and taking into account the depressing influence of surface-active compounds on the fixation, it must be recognized that the catalyst has a two-component structure. The molecule of protein performs in it the function of a colloid carrier, and a low-molecular substance the

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function of the active group. Since the data on the presence of carboxyl, amino, and carbonyl groups in the catalyst's composition could refer to the active ferment group, the question arose about its actual chemical nature. Based on data on the participation of the two adjacent carbonyl groups in the process of fixation, the chemical structure of the compound performing the function of the active group can be determined almost completely.

To obtain nitrogen oxidation with carbonylic oxygen, the presence of a carboxyl group near the carbonyl is of importance. To have the original products even temporarily stabilized, it is necessary to have an amino group next to or in the vicinity of the second carbonyl group, since by blocking the amino with aldehyde the nitrogen fixation is discontinued by reason of the lowered oxidizing activity of the carbonylic oxygen. On the other hand, following the reaction of the amino group with boric or molybdic acid, the fixation increases as a result of the increased oxidizing activity of this oxygen.

Consequently, it should be recognized that the active group of the catalyst of nitrogen fixation has the following structure:

```
R - CH - NH2 - ? - C-C-COOH
                   || ||
                   O  O
```

The nature of the radical R is not known at this stage, but considering the possibility of the active group's reaction with the colloid carrier through the carboxyl group, it can be assumed that the radical represents a second carboxyl group, the active group itself being diketo-amino-glutaric acid. The colloid carrier is connected with this compound. A change in the colloid carrier takes place in the course of fixation. In the first phase, the carrier is formed by protein with an oxidizing potential of about 200 millivolts, and in the second phase by protein with an oxidizing potential of about 300 millivolts.

The catalyst will then have the following structure:

```
Prot.-R-CHNH2-C-C-COOH
              || ||
              O  O
```

The fixation process under participation of the catalyst can be illustrated by the following:

```
1.  Prot1-R-CHNH2-C-C-COOH+N2->Prot1-R-CHNH2-C=C-COOH
                  || ||                      |  |
                  O  O                       O  O
                                             |  |
                                             N==N
```

The first phase of the process ends with this reaction, after which the second phase follows, connected with the reduction of the original fixation products by active hydrogen mobilized from organic matter through the action of dehydrogenase. To complete this phase, a lower oxidation potential is necessary. It is obtained at the expense of replacing the first colloid carrier ($Prot_1$) by the second ($Prot_2$) with a lower oxidizing potential (about 300 millivolts). Because of this decrease in potential, a reaction between the active hydrogen and the original fixation products is possible.

```
2.  Prot2 -R-CHNH2-C=C-COOH+2(Dehydrogenase-H2)->Prot2-R-CHNH2-C=C-COOH
                   |  |                                        |  |
                   O  O                                        C  OH
                   |  |                                        |  |
                   N==N                                        HN-NH2
```

The reduced forms of nitrogen thus obtained, apparently interact with ketonic acids and are transformed into hydrazine derivatives of the corresponding ketonic acids:

$$3.\quad \mathrm{Prot_2} - \mathrm{R\text{-}CHNH_2\text{-}}\underset{\substack{| \\ \mathrm{O} \\ | \\ \mathrm{HN}}}{\mathrm{C}}\text{-}\underset{\substack{| \\ \mathrm{OH} \\ | \\ \text{-}\mathrm{NH_2}}}{\mathrm{C}}\mathrm{\text{-}COOH+R\text{-}}\underset{\substack{| \\ \mathrm{COOH}}}{\mathrm{CO}} \rightarrow \mathrm{Prot_2} - \mathrm{R\text{-}CHNH_2\text{-}}\underset{\substack{| \\ \mathrm{OH}}}{\mathrm{C}}\text{-}\underset{\substack{\backslash \\ \mathrm{OH}}}{\mathrm{C}}\mathrm{\text{-}COOH+R\text{-}}\underset{\substack{| \\ \mathrm{COOH}}}{\mathrm{C}}\mathrm{:N.NH_2}$$

Hydrazine derivatives of ketonic acids are reduced to amino acids and used for the building up of the protein of the Azotobacter's protoplasm:

$$4.\quad \mathrm{R\text{-}}\underset{\substack{| \\ \mathrm{COOH}}}{\mathrm{C}}\mathrm{:N\text{-}NH_2+R\text{-}}\underset{\substack{| \\ \mathrm{COOH}}}{\mathrm{CO}}\mathrm{+3\,(dehydrogenase\text{-}H_2)} \rightarrow \mathrm{2R\text{-}}\underset{\substack{| \\ \mathrm{COOH}}}{\mathrm{CHNH_2}}\mathrm{+H_2O}$$

All factors connected with the fixation of atmospheric nitrogen by bacteria are explained by the above method. The positive effect of oxygen, hydrogen peroxide, and other oxidizing agents is due to the formation of free carbonyl groups in the catalyst for nitrogen fixation, while the depressive effect of molecular hydrogen, hydroquinone, hydrogen sulfide, and other reducing substances is due to the reduction of carbonyl groups.

The depressive effect of aldehydes reacting with the amino group is connected with the introduction into the molecule of active groups of reduced compounds. The latter interfere with the oxidation of nitrogen and change the chemical properties of the catalyst. The activating effect of boric and molybdic acids is based on the introduction of oxidizing compounds favoring the oxidation of nitrogen into the molecule of the active group of the catalyst. The depressing effect of surface-active substances can be explained by the blocking of border surfaces between the active group and the colloid carrier, and the depressive action of heavy metals by the change of the state of the active group as well as of the colloid carrier.

All facts are thus clarified and the possibility of partially removing fixation products by means of solution is a direct confirmation of the possible formation of hydrazines as original products of atmospheric nitrogen binding.

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